Investigator: Richard Sayre

Title: OPTIMIZATION OF BIOFUEL PRODUCTION FROM TRANSGENIC MICROALGAE

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Chlorella transformation: We have developed nuclear transformation vectors based on the Ble resistance gene. We observed that Chlorella was resistant to a number of antibiotics that other algae were sensitive too. This necessitated a screen for the best antibiotic resistance genes to use for transformation selectable markers. Using the Chlamydomonas ss-rbcl and psaD promoters we have obtained transgenic Chlorella cells at high frequency that have been confirmed by PCR analysis. We also have now completed the genome sequence of the actin gene and its promoter from C. prothecoides. This promoter is now being incorporated into a transformation vector.

**Optimization of light harvesting antennae size:** Typically, wild type algae light saturate at 1/3 of full sunlight intensity. This is associated with an over-efficient light harvesting chlorophyll a/b (LHC) complexes that light saturate the reaction centers. By reducing the antennae size it has been shown in the lab that it is possible to increase photosynthetic quantum efficiency.

Using two independent strategies we have successfully altered the chlorophyll a/b ratio, and as demonstrated by chlorophyll fluorescence induction kinetics, the LHC antennae size of transgenic algae expressing either a chlorophyll a oxidase RNAi construct or by over-expressing chlorophyll b reductase. Wild type algae have chlorophyll a/b ratios of 2. The transgenics have chlorophyll a/b ratios approaching 4 indicative of a 90% reduction in chlorophyll b content. We are currently planning to do high light (full sunlight) growth experiments to determine empirically the best LHC content for optimal growth at high light intensities.

Non-destructive oil extraction: Harvesting algae and extracting oil accounts for 50% of the cost of producing algal biofuels. Much of this cost is associated with concentrating algae from 0.1% of the mass of the pond to nearly 80% of the extractable material. This dewatering process is energetically very expensive. Also, algae are currently destroyed during the extraction process so a new culure must be grown each time the algae are harvested. A more efficient oil extraction technology would eliminate or substantially reduce the dewatering and non-destructively extract oils so the cultures would not need to be re-grown. The biocompatible solvent system we have developed meets these criteria. Using a variety for short, straight-chain alkanes and optimal mixing procedures we have been able to extract quantitatively neutral lipids from Chlorella and Nanochloropsis. We have gone as many as five extraction cycles with nanochloropsis with no apparent reduction in growth. We have submitted a patent on this process.

Metabolic Engineering: We are focusing on engineering both lipid synthesis and photosynthetic carbon fixation to optimize oil production. Gene constructs have been developed to engineer pyruvate metabolism in algae and are ready to transform. We are nearing completion of a Rubisco construct linked to carbonic anhydrase to increase the CO2 concentration near the active site of Rubisco to inhibit photorespiration. In addition, we have started a project on characterizing the proteomes of cells induced to produce oils under different conditions. These studies are expected to provide insights into which genes to target for enhanced oil production.

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